



Diachronic adjustments of functional traits scaling relationships to track environmental changes: revisiting *Cistus* species leaf cohort classification[☆]

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ABSTRACT

Leaf functional traits and their relationships can differ between leaf flushes, particularly for species characterized by an extended growing season such as Mediterranean ones. Among them, *Cistus* spp. are generally reported to display two different leaf cohorts (i.e. summer and winter leaves) during the same growing season. We tested the generality of such leaf cohort classification by analyzing the diachronic adjustments of relationships between different leaf functional traits in 3 *Cistus* spp. Traits included were: leaf mass per area (LMA), leaf thickness (LT), leaf dry matter content (LDMC) and net photosynthesis on a mass basis (Am). The slopes of the relationships between morphological traits were then regressed against climate variables. The slopes were also regressed against leaf production rate (LPR) and the scores of the positioning as Competitors (%C), Stress tolerators (%S) and Ruderals (%R) in the tertiary CSR scheme.

The different leaf flushes reshaped the well-known trait covariation patterns thus reflecting shifts in leaf-level resource-acquisition and -use strategies. This was achieved through an opposite response of the relationships LMA–LT and LMA–LDMC to temperature changes. In fact, the relationship LMA–LT was better modulated in leaves produced at different temperatures, while the LMA–LDMC one was possibly constrained due to the tighter negative relationship LDMC–Am (i.e. higher costs in terms of carbon assimilation). Accordingly, changes in LMA–LDMC coordination were significantly related to %C.

Our results provide an evidence that while *Cistus* spp. leaf cohort classification generally holds, it does not necessarily capture the entire seasonal spectrum of traits covariation. We propose to move forward from the generally accepted winter–summer leaf classification shown in literature providing a new framework that can better describe *Cistus* species potential response to environmental changes.

1. Introduction

Species characterized by an extended growing season generally display a complex phenology of foliage development with multiple leaf flushes (Niinemets, 2014). As such, leaf cohorts are formed under differing environmental conditions and major differences in leaf traits depending on flushes exist (Morales et al., 2014). Niinemets (2014) also argued that leaf flushes may be characterized by differences in trade-off rates between functional traits compared to that predicted by the Leaf Economic Spectrum (Wright et al., 2004). However, whether leaf cohorts can be considered as a source of variation of trait–trait relationships has been poorly investigated, with few exceptions (e.g. Puglielli and Varone, 2018), mainly because of the difficulty to collect data on different leaf cohorts in a wide range of species and environmental

conditions.

These aspects are particularly interesting for Mediterranean species, which are usually characterized by an extended growing season with strong climatic fluctuations. Among them, *Cistus* spp. developed under Mediterranean climate and are determinants of composition and current diversity of the Mediterranean area (Gratani and Varone, 2004; Correia and Ascensão, 2017). *Cistus* spp. are key components of the Mediterranean ecosystems due to their pioneer character being able to act as a source of nutrients to the soil which facilitate vegetation succession after disturbance (Simões et al., 2009; de Dato et al., 2013). They can in fact enrich top soil layers of organic carbon and improve nutrient status in general through deposition of litter under the canopy and leaching from green leaves and litter. Also, they can fasten P cycle, even if the rate can depend on the species (Simões et al., 2009).

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Cistus genus is a good candidate to test leaf cohort dependent changes in functional traits. The 21 species belonging to this genus are considered as seasonal dimorphic semideciduous shrubs which display two well defined leaf cohorts during the same growing season, the so-called summer and winter leaves (Aronne and De Micco, 2001). Summer and winter leaves significantly differ in anatomical, morphological and physiological leaf traits (Aronne and De Micco, 2001; Catoni et al., 2012) and indeed display different strategies to face the environmental cues of the Mediterranean climate (Puglielli and Varone, 2018). Additionally, Puglielli et al. (2017a) highlighted that the relationship between LMA and the photosynthetic rate per unit of leaf mass (Am), as well as the relationships between LMA and its anatomical components (leaf tissue density and thickness; Poorter et al., 2009), followed seasonal changes in three *Cistus* spp. from different provenances. This evidence increases the rationale to test for the existence of functionally different leaf populations (i.e. leaf cohorts) along a particular growing season.

The present work is therefore a follow up of Puglielli et al. (2017a). In fact, we used this published dataset implemented with original data from a common garden experiment, being the latter already proved as a means to test for leaf cohort dependent changes in functional traits (Morales et al., 2014). Our aim was to test the generality of the classification of *Cistus* leaves in winter and summer leaves (Aronne and De Micco, 2001). We hypothesized that *Cistus* spp. can produce leaves characterized by different acclimatory capacity during a growing season and that winter and summer leaves represent the extremes of such continuum. Acclimatory capacity of leaves formed under different environmental conditions was determined by the slopes of the bivariate relationships between functional traits that have been already proved to be suitable means to test for leaf cohort acclimation to varying environmental conditions (see Atkinson et al., 2010 and references within). As such, differences in scaling relationships have been observed in comparisons of plants grown in sites differing in nutrients and rainfall levels (Wright et al., 2001), in rainfall and mean annual temperature (Wright et al., 2005) as well as in plants grown under ambient and elevated atmospheric CO₂ concentration (Tjoelker et al., 1999).

Solving leaf cohort classification in *Cistus* spp. has important consequences for the understanding of their ability to respond to environmental changes. In fact, a better knowledge on the leaf flush-dependent strategy to cope with climate variations can improve the prediction of the response of these species in the face of climate change.

2. Materials and methods

2.1. Plant material and data collection

For this experiment we used potted *Cistus monspeliensis* L., *C. salvifolius* L. and *C. creticus* subsp. *eriocephalus* (Viv.) Greuter & Burdet (i.e. the most widespread species of the genus) plants grown from seeds of different provenances. In particular, *C. monspeliensis* seeds were collected in South Spain (Loc. Pantano Quebrajano, 37° 37' 57.7" N; 03° 43' 43.9" W, Andalucía) and in Sardinia (Loc. Guspini, 39° 32' 32" N; 8° 38' 02" E, Medio Campidano). *Cistus salvifolius* seeds were collected in S-W Spain (Loc. Huelva, 37° 15' 41" N; 6° 57' 54" W, Andalucía) and in Sardinia (Loc. Portixeddu, 39° 26' 32" N; 8° 24' 37" E, Carbonia-Iglesias). *Cistus creticus* subsp. *eriocephalus* seeds were collected in Sardinia (Loc. Portixeddu, 39° 26' 32" N; 8° 24' 37" E, Carbonia-Iglesias) and on the Italian mainland (Loc. Foce del Garigliano, 41° 13' 23" N; 13° 45' 45" E, Caserta). The choice of CE seeds from the Italian mainland is because the species is not present in Spain.

Plants were grown in 101 pots containing an organic commercial substrate (COMPO BIO, COMPO GmbH, Germany) with the following composition: organic carbon (C) 35%, humic carbon 11%, organic nitrogen (N) 1.4%, carbon on total nitrogen ratio of 25, peat (65%) and pH(H₂O) 6.0–7.0. Plants were not fertilized during the experiment that was carried out under natural conditions inside the Botanical Garden of

Sapienza University of Rome (41° 53' 33" N, 12° 27' 54" E, 17 m a.s.l.) where plants grow under a Mediterranean type of climate.

Concerning the sampling design, thirty-six three-years-old plants (mean height 62 ± 12 cm) were arranged in a completely randomized design (CRD) with six replicates per species and provenance (i.e., replicates assigned completely at random to independent experimental subjects), and watered to prevent drought stress in the absence of rainfall. In order to test for differences in acclimation of leaves to changing environmental conditions, we first marked ten branches (in the upper portion of the shrubs) per provenance and then followed leaf evolution. The leaves were considered fully expanded when they reached a horizontal position (i.e., ca. 0°; Oliveira and Peñuelas, 2002). Measurements were thus carried out on 6 fully expanded apical leaves (one leaf per plant per each species and provenance) randomly selected among the marked branches in the period March–July 2017.

In order to include data for an entire growing season we combined the data obtained in the period March–July 2017 with an already published dataset (Puglielli et al., 2017a) that covered the period from November 2015 to April 2016. The two datasets had overlapped the time period March–April and a preliminary analysis (data not shown) highlighted that the bivariate relationships between the considered leaf traits in the period March–April during the two growing seasons did not differ in terms of slopes. Such result allowed us to merge the two datasets. The final dataset included the period EPI to EPIV (from mid-November to the beginning of April) from Puglielli et al. (2017a) and original data for EPV (from mid-April to the beginning of May) and EPVI (from mid-June to the beginning of July), thus covering an entire growing season.

As in Puglielli et al. (2017a), we obtained data for mean, mean maximum and mean minimum air temperature (T_{mean}, T_{max} and T_{min}, °C), global radiation (GR, Watt m⁻²) and photosynthetic photon flux density (PPFD, μmol m⁻² s⁻¹) relative to EPV and EPVI from the Meteorological Station inside the Botanical Garden of Rome (Table 1).

On each sampling occasion during the period May–July 2017 we measured leaf mass per area (LMA, g m⁻²), leaf thickness (LT, μm), leaf dry matter content (LDMC, mg g⁻¹) according to standard protocols (Cornelissen et al., 2003; but see also Puglielli et al., 2017a). We also determined photosynthesis per unit of leaf mass (Am, nmol CO₂ g⁻¹ s⁻¹) by the ratio between net photosynthesis on area basis (Aa, μmol CO₂ m⁻² s⁻¹) and LMA. Aa measurements were performed between 9 and 12 a.m. (Gratani and Varone, 2004) using an infrared gas analyser in an open system (LCpro +; ADC, Hoddesdon, UK), equipped with a 6.25 cm² leaf chamber (PLC, Parkinson leaf chamber). Measurements were carried out at ambient CO₂ of 400 μl l⁻¹. Leaf temperature was kept at ambient temperature and varied by 1% during measurements in each EP. Relative humidity was between 50 and 60% during measurements. Photosynthetic photon flux density (PPFD) of 1500 μmol m⁻² s⁻¹ was provided by the light source (LCpro + Lamp unit; ADC). The number of leaves produced in each EP was also recorded for each marked branch and Leaf Production Rate (LPR, n n⁻¹ day) was

Table 1

Climate characteristics of the experimental periods (EP): mean minimum daily air temperature (°C), mean maximum daily air temperature (°C), mean daily air temperature (°C), global radiation (GR, Watt m⁻²), photosynthetic photon flux density (PPFD, μmol m⁻² s⁻¹).

EP	Temperature			GR	PPFD
	Min	Max	Mean		
I	5.79	17.25	10.15	485.9	313.1
II	3.75	15.16	7.95	617.7	286.9
III	6.21	17.40	10.99	421.4	468.7
IV	7.27	20.35	12.70	571.4	749.3
V	19.62	21.36	20.50	530.0	997.5
VI	29.53	31.18	30.35	588.5	1140.2

calculated according to [Cochrane et al. \(2015\)](#) as the natural log of the difference between the number of leaves at the beginning and at the end of each EP divided by the length of the time interval (days).

2.2. Data analysis

Preliminary analysis revealed a very weak signal of intraspecific variability for all the considered traits (most of the effect was due to differences between provenances of different species) and differences in traits between experimental periods were higher compared to the differences between species (F values main effects = 30.94 and 7.50, respectively). Thus, data were pooled across species in order to reveal general response patterns at the genus level. One-way ANOVA was carried out in order to test for significant differences in the considered leaf traits between EPs at $p \leq 0.05$. Multiple comparisons were analyzed by Tukey test.

Standardized major axis regression (SMA; [Warton et al., 2006](#)) was used to analyze the considered log–log bivariate relationships. Concerning the grouping variable, *Experimental Period* (six levels EPI–VI) was used. Differences in terms of slopes were tested with the Likelihood Ratio statistic (see [Warton et al., 2006](#) for further details). If pertinent, differences between intercepts were tested with the Wald statistic. Multiple comparisons were always performed after adjusting p-values with the Sidak correction ([Warton et al., 2012](#)).

In order to select environmental variables to be regressed against slopes of the bivariate relationships, daily climatic data were combined through Principal Component Analysis (PCA) in a correlation matrix in order to define the major axis of climate variations (i.e. first principal component, PC1, Supplementary Fig. 1 and Table 1). The variables significantly related to PC1 were used in subsequent analyses. Since the relationships were far from linearity, non-linear models were fitted on the data by using the *nls* function of the *nls2* R package ([Grothendieck, 2013](#)). In non-linear relationship analysis we used the slopes of the bivariate relationships between morphological traits which were significant within each EP. The choice to use only the slopes of the bivariate relationships between morphological traits is due to the rationale that variability in relative changes (i.e. slopes) of LMA as a function of LT and LDMC, as well as the relative changes of LDMC as a function of LT, provides insights on changes in species resource-acquisition and -use strategies ([Niinemets, 1999](#); [Puglielli et al., 2015](#); [Griffith et al., 2016](#)). The relationships between leaf morphological traits and Am were treated separately. Correlation analysis was also carried out to test for the relationships between mean values of the considered morphological traits and the climatic variable selected through PCA.

To further test our hypothesis, simple linear regression analysis was used to test the relationship between the slopes of the considered bivariate relationships and LPR across EP. Moreover, by implementing the methodology proposed by [Pierce et al. \(2013\)](#), we used data of leaf area (LA), specific leaf area (SLA = $1/\text{LMA}$) and LDMC in order to quantify changes in *Cistus* spp. strategies as Competitors, Stress tolerators and/or Ruderals (CSR) according to the CSR scheme ([Grime, 1977](#)) across EPs. The obtained scores (hereafter %C, %S and %R) were then regressed against the estimated slopes in order to explicitly relate them to changes in species resource-acquisition and -use strategies.

3. Results

Among the considered morphological traits, LMA, LT and LDMC did not significantly vary between EPI and EPIII even if a tendency to higher values for all the parameters was observed in EPII (Fig. 1A–C). LMA and LT increased from EPIII to EPIV ($p < 0.05$) and then decreased in EPV ($p > 0.05$) (Fig. 1A,C) while LDMC slightly decreased from EPIII to EPIV ($p > 0.05$) and from EPIV to EPV ($p < 0.05$) (Fig. 1B). LMA, LDMC and LT were characterized by significantly higher values in EPVI (Fig. 1A–C). Am did not show significant

differences from EPI to EPIII and it decreased ($p < 0.05$) in EPIV. Am showed the highest and the lowest values in EPV and EPVI, respectively (Fig. 1D).

The bivariate relationships LMA–LDMC and LMA–LT were significant within each EP with R^2 values ranging between 0.11–0.81 and 0.37–0.90, respectively (Table 2), reflecting the general relationships found across EPs (Fig. 2A,B). Conversely, LDMC and LT were weakly coordinated on pooled data (Fig. 2C) and their relationship was in fact significant only in EPI, EPIV and EPVI (Table 2). The morphological control on the photosynthetic process (Am vs LMA, LT and LDMC) was highly significant on pooled data across EPs (Fig. 2D–F). Nevertheless, such relationships were mostly significant from EPIV to EPV (Table 2). Moreover, significant differences in slopes between the considered (significant) bivariate relationships were found (Table 2).

PCA carried out on climate data revealed that changes in air temperature (Tmean, Tmax and Tmin) changes captured most of the variance between EPs (Supplementary Fig. 1 and Table 1). In fact, they were highly correlated with PC1 which explained 71% of variance between EPs. Therefore, Tmean, Tmax and Tmin were used in slope–climate relationship analyses.

LMA and LT were correlated with Tmean, Tmax and Tmin ($R = 0.79$ – 0.90 and 0.83 – 0.89 , $p < 0.05$) while LDMC was not ($R = 0.40$ – 0.55 , $p > 0.05$) (Table 3). However, the slopes of the relationships LMA–LT and LMA–LDMC (as well as the pooled slopes) were non-linearly related to changes in air temperature (Table 4). The analysis carried out on pooled slopes revealed a higher coordination of slope changes with Tmin ($R = 0.69$) and Tmean ($R = 0.67$) compared to Tmax ($R = 0.64$) (Table 4). The same pattern was evident when the slopes of LMA–LT and LDMC–LT were considered separately (Table 4) even if slopes of LMA–LT displayed a higher correlation with Tmin, Tmean and Tmax (average $R = 0.95 \pm 0.05$) compared to the slopes of LMA–LDMC (0.80 ± 0.02), for which no significant coefficients were found (Table 4). The slopes of the relationships LDMC–LT were excluded from this analysis since they were not significant within each EP.

Ultimately, the pooled slopes of LMA–LT and LMA–LDMC were significantly related to changes in %C ($R^2 = 0.40$, $p < 0.05$) (Fig. 3A). However, even if the same pattern was observed when the slopes of LMA–LDMC and LMA–LT were considered separately, the slopes of LMA–LDMC showed a tighter coordination with %C ($R^2 = 0.78$, $p < 0.05$) while the relationship between %C and the slopes LMA–LT was not significant ($R^2 = 0.48$, $p > 0.05$) (Fig. 3B,C). Conversely, the relationship between the pooled slopes of LMA–LT and LMA–LDMC with LPR was not significant ($R^2 = 0.16$, $p > 0.05$) (Fig. 3D). When the slopes pools were considered separately, then the slopes LMA–LT were significantly related to changes in LPR ($R^2 = 0.69$, $p < 0.05$) while the slopes LMA–LDMC were not ($R^2 = 0.13$, $p > 0.05$) (Fig. 3E,F).

4. Discussion

Our results showed that leaves formed from November to March (i.e. from EPI to EPIII, winter leaves) tend to be characterized by not significant differences. On the other hand, leaves formed between June and July (i.e. EPVI) are characterized by significant differences compared to the rest of the leaves sampled through EPs. In fact, they are characterized by significantly higher values of LMA, LDMC and LT reflecting the well-known features of summer leaves ([Aronne and De Micco, 2001](#); [Puglielli and Varone, 2018](#)). Moreover, a tendency to higher values for all the considered morphological traits was observed under chilling (in EPII), reflecting some kind of leaf hardening as already described in previous studies ([Gratani and Bombelli, 1999](#); [Gratani and Varone, 2004](#); [Oliveira and Peñuelas, 2004](#)). Also, significant differences for the considered morphological traits during the favorable period comprised between April and May (i.e. EPIV and EPV) were observed. Significant differences in functional traits of *Cistus* spp. leaves under more favorable conditions have been already observed in

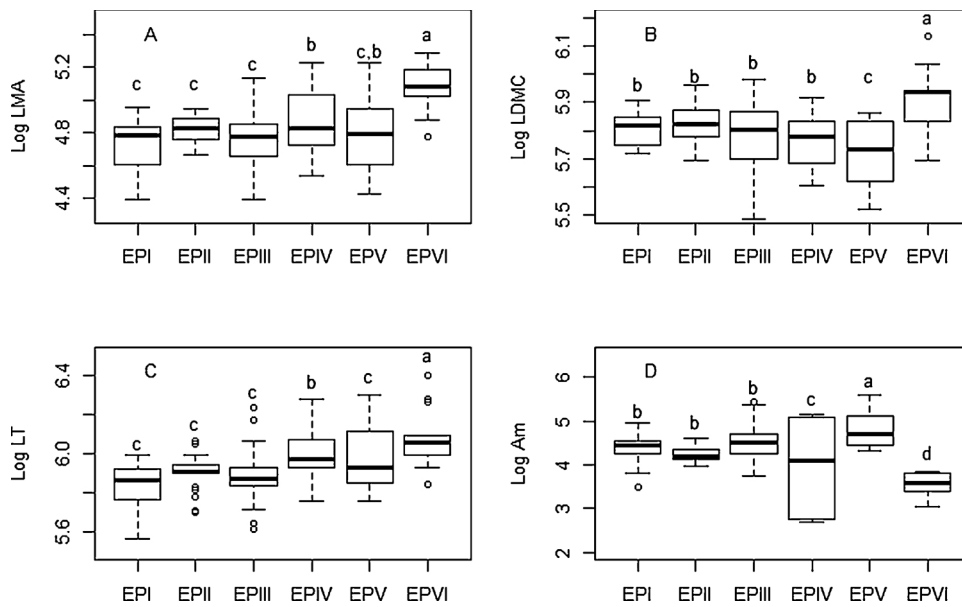


Fig. 1. Median values and the 10th and 90th percentile of the distribution for the log-transformed: (A) leaf dry mass per unit area (LMA), (B) leaf dry matter content (LDMC), (C) leaf thickness (LT) and (D) photosynthesis per unit of leaf mass (Am) for each Experimental Period (EP, n = 36). Different letters indicate significant differences between EPs (One-way ANOVA, $p \leq 0.05$).

Table 2

Log-log relationships between the considered parameters. LMA = leaf dry mass per unit area (g m^{-2}), LT = leaf thickness (μm), LDMC = leaf dry matter content (mg g^{-1}), Am = photosynthesis per unit of leaf mass ($\text{nmol CO}_2 \text{g}^{-1} \text{s}^{-1}$). Slope, intercept, lower and upper limit (in parenthesis), R^2 and p value per each relationship and Experimental Period (EP) are given. n = 36. Slopes with different letters were statistically different at $p \leq 0.05$.

Relationship	EP	Slope		Intercept		R ²	p value
LMA–LDMC	I	2.53a	(1.99,3.19)	−9.93	(−13.40,−6.47)	0.54	3.22E-07
	II	1.11b	(0.80,1.53)	−1.65	(−3.78,0.47)	0.11	0.046
	III	1.59b	(1.33,1.89)	−4.43	(−6.05,−2.80)	0.58	8.89E-12
	IV	2.44a	(2.10,2.85)	−9.23	(−11.38,−7.07)	0.81	1.04E-13
	V	1.90b	(1.37,2.63)	−6.06	(−9.65,−2.46)	0.44	0.0004
	VI	1.22b	(0.84,1.77)	−2.15	(−2.70,0.93)	0.15	0.048
LMA–LT	I	1.32b	(1.16,1.50)	−2.97	(−3.94,−1.10)	0.87	< 2.22e-16
	II	0.95c	(0.72,1.24)	−0.77	(−2.32,0.77)	0.37	0.00007
	III	1.51b	(1.28,1.79)	−4.13	(−5.63,−2.63)	0.62	6.21E-13
	IV	1.54a	(1.38,1.72)	−4.40	(−5.42,−3.37)	0.90	< 2.22e-16
	V	1.31b	(1.05,1.65)	−3.06	(−4.86,−1.26)	0.73	1.05E-07
	VI	0.98c	0.73,1.32	−0.88	(−2.70,0.93)	0.45	0.00012
LDMC–LT	I	0.52b	(0.38,0.71)	2.75	(1.80,3.70)	0.20	0.006
	II	−0.85a	(−1.19,−0.61)	10.85	(9.15,12.55)	0.06	0.156
	III	0.95a	(0.73,1.24)	0.19	(−1.31,1.68)	0.04	0.137
	IV	0.63b	(0.51,0.79)	1.97	(1.13,2.82)	0.59	3.81E-08
	V	0.69b	(0.45,1.06)	1.58	(−0.22, 3.38)	0.03	0.408
	VI	−0.80a	(−1.15,−0.56)	10.77	(8.96,12.59)	0.18	0.027
LMA–Am	I	−2.54b	(−3.57,−1.80)	16.38	(12.19,20.57)	0.0005	0.898
	II	−2.21b	(−2.99,−1.64)	14.89	(11.64,18.13)	0.002	0.239
	III	−2.26b	(−2.82,−1.81)	15.26	(12.85,17.68)	4.83E-06	0.323
	IV	−4.76a	(−5.55,−4.08)	27.08	(23.51,30.65)	0.80	1.44E-13
	V	−1.70c	(−2.23,−1.30)	12.94	(10.69,15.18)	0.61	6.04E-06
	VI	−1.72c	(−2.55,−1.16)	12.30	(8.78,15.82)	0.04	0.313
LT–Am	I	3.35b	(2.38,4.71)	−15.20	(−21.99,−8.40)	0.007	0.626
	II	−2.10c	(−2.94,−1.49)	16.60	(12.32,20.88)	0.018	0.428
	III	−3.42b	(−4.41,−2.65)	24.60	(19.41,29.78)	0.014	0.106
	IV	−7.34a	(−8.97,−6.01)	48.00	(39.15,56.86)	0.667	1.24E-09
	V	−2.23c	(−3.28,−1.52)	18.14	(12.88,23.39)	0.208	0.025
	VI	1.68c	(1.13,2.50)	−6.65	(−10.81,−2.48)	0.024	0.44
LDMC–Am	I	−6.41b	(−8.92,−4.60)	41.57	(29.05,54.09)	0.067	0.124
	II	−2.46d	(−3.37,−1.80)	18.54	(13.96,23.12)	0.016	0.158
	III	−3.59c	(−4.49,−2.87)	25.27	(20.58,29.95)	6.87E-06	0.315
	IV	−11.63a	(−13.79,−9.81)	70.98	(59.51,82.45)	0.76	4.93E-12
	V	−3.23c	(−4.13,−2.52)	23.24	(18.64,27.85)	0.68	6.76E-07
	VI	−2.10d	(−3.02,−1.46)	16.00	(11.41,20.59)	0.19	0.021

previous studies (e.g. Werner and Máguas, 2010; Catoni et al., 2012; de Dato et al., 2013).

What we do not know is if only the differences in mean values of functional traits can be considered a good proxy of changes in

acclimation of *Cistus* spp. leaves to changing environmental conditions. According to Atkinson et al. (2010), a similar acclimatory capacity of leaves developed at different temperature treatments resulted in the lack of differences in slopes for the scaling relationships between leaf

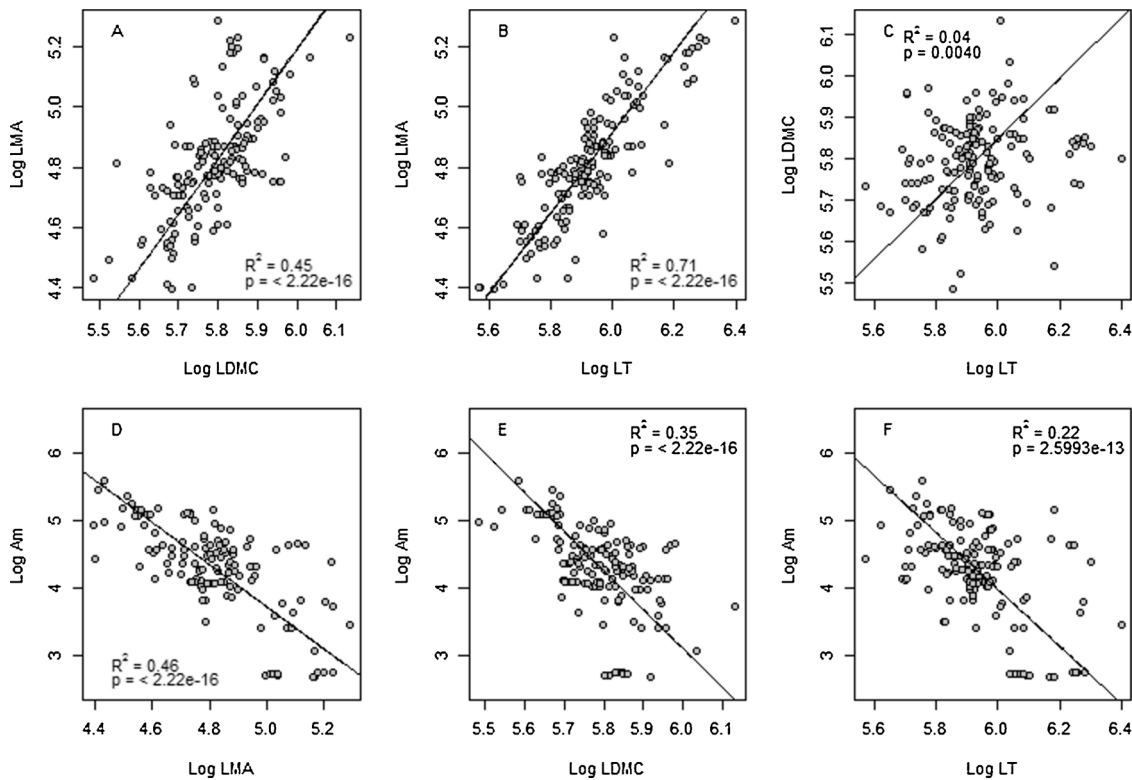


Fig. 2. Log-log relationships ($n = 216$) between: (A) LMA–LDMC, (B) LMA–LT, (C) LDMC–LT, (D) Am–LMA, (E) Am–LT and (F) Am–LDMC across Experimental Periods. LMA = leaf dry mass per unit area, LDMC = leaf dry matter content, LT = leaf thickness, Am = photosynthesis per unit of leaf mass. R^2 and p are also shown.

Table 3

Pearson pair-wise correlation coefficients between leaf mass area (LMA, g m^{-2}), leaf thickness (LT, μm), leaf dry matter content (LDMC, mg g^{-1}) and mean minimum daily air temperature (Tmin, $^{\circ}\text{C}$), mean maximum daily air temperature (Tmax, $^{\circ}\text{C}$), mean daily air temperature (Tmean, $^{\circ}\text{C}$). Mean values within each Experimental Period (see text) were used as experimental units ($n = 6$). Values in bold denotes significance at $p \leq 0.05$.

	Tmin	Tmax	Tmean
LMA	0.79	0.90	0.82
LT	0.83	0.89	0.86
LDMC	0.40	0.55	0.43

Table 4

Coefficients, sample size (n), standard errors of the model (SE) and correlation coefficient (R) of the non-linear model (in the form $y = a + bx + c/x^2$) used to describe variation of the estimated slopes for the log-log relationships LMA–LDMC and LMA–LT for each Experimental Period (EP) against mean minimum daily air temperature (Tmin, $^{\circ}\text{C}$), mean daily air temperature (Tmean, $^{\circ}\text{C}$) and mean maximum daily air temperature (Tmax, $^{\circ}\text{C}$). LMA = leaf dry mass per unit area (g m^{-2}), LDMC = leaf dry matter content (mg g^{-1}), LT = leaf thickness (μm). Bold coefficients were significant at $p < 0.05$.

Slopes		a	b	c	n	SE	R
Pooled	Tmin	2.660	−0.052	−20.221	12	0.42	0.69
	Tmean	3.726	−0.084	−124.434	12	0.43	0.67
	Tmax	7.684	−0.184	−862.610	12	0.44	0.64
LMA–LDMC	Tmin	3.301	−0.069	−27.018	6	0.43	0.83
	Tmean	4.688	−0.110	−163.757	6	0.46	0.80
	Tmax	10.117	−0.248	−1170.328	6	0.48	0.78
LMA–LT	Tmin	2.019	−0.034	−13.424	6	0.07	0.98
	Tmean	2.763	−0.057	−85.111	6	0.07	0.97
	Tmax	5.251	−0.119	−554.892	6	0.15	0.89

functional traits. This is also in agreement with the ‘shift 1’ scenario proposed by Wright et al. (2001) which predicts that a similar acclimatory capacity should be highlighted by a proportional x–y scaling in order to maintain a constant slope. Since we found differences in slopes for the considered (significant) bivariate relationships, this evidence highlights that leaves produced under different environmental conditions in *Cistus* spp. do display a different acclimation capacity, thus modifying the commonly found trait covariation patterns (Fig. 2; Table 2). This is consistent with previous studies that have shown how changing environmental factors can reshape the well-known trait correlation networks (Lusk et al., 2008; Hallik et al., 2009; Niinemets, 2015, 2016; González-Zurdo et al., 2016).

Also, changes in slopes of the considered bivariate relationships as a function of temperature roughly display a temperature-like response curve, opposite to the linear increase observed for LMA and LT, and the lack of correlation of LDMC, with temperature changes. Thus, leaf covariation patterns between morphological traits behave differently from mean values and, when they are accounted for, shifts in species strategies (i.e. differences in slopes) are more consistent than on the basis of mean values only. This statement is also confirmed by the analysis of the morphological control of the photosynthetic process. In fact, we observed a clear shift from a lack of coordination between morphological traits and Am (from EPI to EPIII, winter leaves) to significant relationships under more favorable conditions (the highest R^2 in EPIV and EPV). A reduced extent of morphological control on the photosynthetic process in the so-called winter leaves compared to summer leaves has been recently documented (Puglielli and Varone, 2018). The authors found that winter and summer leaves are characterized by a high and low return strategy (*sensu* Wright et al., 2004), respectively. Hence, winter leaves tend to maximize resource acquisition with a lower investment in dry mass, while summer leaves adopt an opposite strategy. These differences are reflected by differences in leaf life span between these cohorts, ≈ 6 and ≈ 10 months for winter

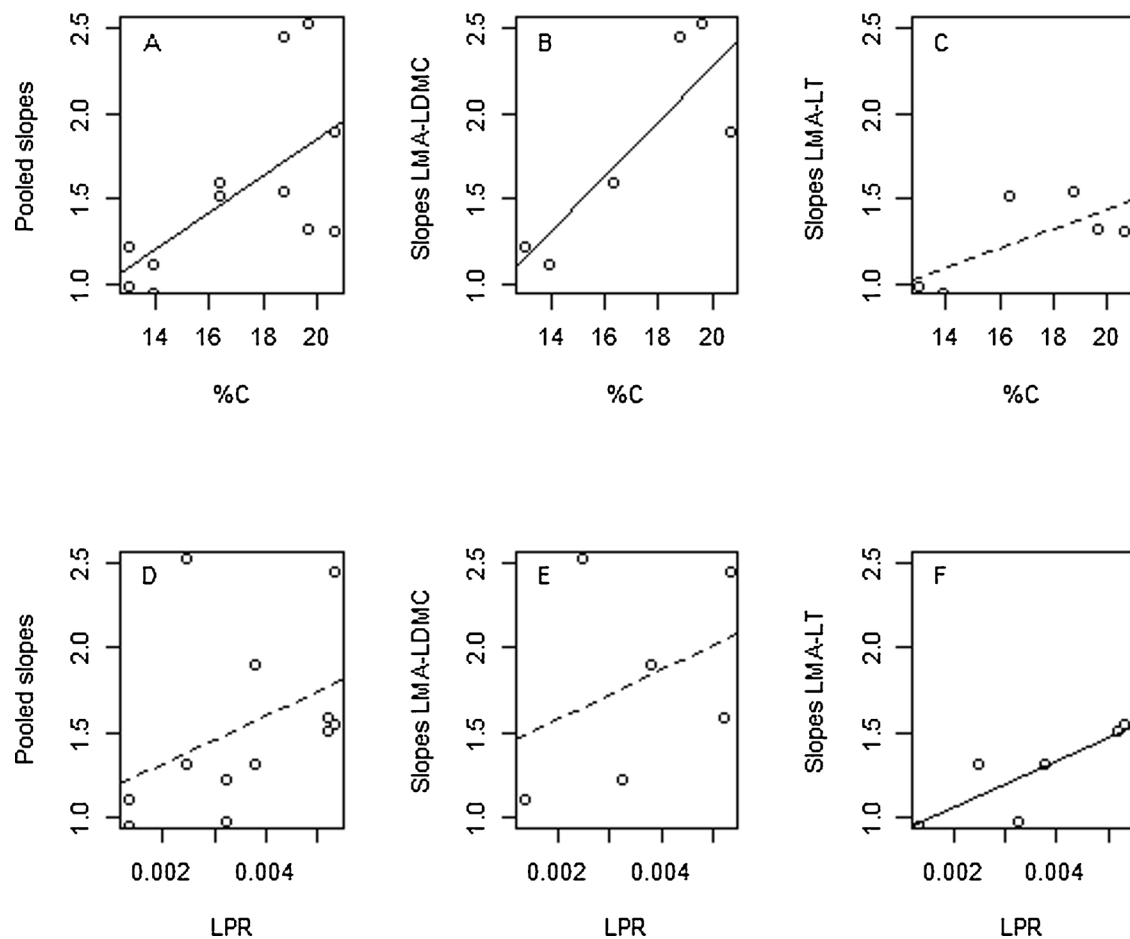


Fig. 3. Relationships between slopes of the bivariate relationships LMA–LDMC and LMA–LT and: (A–C) the estimated scores as competitors (%C) and (D–F) mean leaf production rate across species (LPR). Continuous lines indicate significant relationships at $p < 0.05$ while dashed line no significant relationships. LMA = leaf dry mass per unit area, LDMC = leaf dry matter content, LT = leaf thickness.

and summer leaves, respectively (Correia et al., 1992; Gratani and Bombelli, 1999; Oliveira and Peñuelas, 2000).

Indeed, shift in strategies between *Cistus* spp. leaf cohorts are possible at the extremes of a growing season. Accordingly, species characterized by an extended period of leaf production, such as *Cistus* spp. (Supplementary Fig. 2; but see also Gratani and Crescente, 1997; Werner and Máguas, 2010), have been reported to be subjected to environmentally driven morphogenesis of expanding leaves (Abrams and Kubiske, 1990; Wyka et al., 2012; Morales et al., 2014). Thus, the different leaf cohorts can acclimate functional traits, and their relationships, during a growing season (Clane and Harrington, 2007; Morales et al., 2014; Niinemets, 2014). Our results extend this view to *Cistus* spp. by pointing out that the shift from winter to summer leaf strategy (Puglielli and Varone, 2018) has, at least, another intermediate stage during the transition from winter–early spring (EPII–EPIII) to more favorable environmental conditions (EPIV–EPV).

Concerning the changes in LMA as a function of its anatomical components, we found that changes in LMA are mainly due to LT rather than LDMC according to previous studies (e.g. Griffith et al., 2016) and such pattern was consistent both across and within EPs. Accordingly, changes in the slopes of the relationship LMA–LT showed higher correlation coefficients with temperature changes through EPs than the slopes LMA–LDMC. This result is complemented by the significant correlation between slopes of LMA–LT and LPR, which is indeed a function of temperature. On the other hand, the modulation of LDMC as component of LMA in new formed leaves is basically independent of LT changes, as attested by the weak coordination for LT–LDMC found both across and within EPs. The weak, or even the lack of, coordination

between LT and LDMC has been often reported in the literature (Niinemets, 1999; Griffith et al., 2016; Puglielli et al., 2015, 2017b; Gratani et al., 2018). This result agrees with the concept of leaf sub-modules proposed by Li et al. (2017) which implies the semi-independence of modules carrying a different function, such as light interception, water–nutrient flow and gas exchange. From this point of view, LT changes can be associated to structural changes such as the number of cell layers (i.e. light interception), whereas LDMC is associated with concentrations of photosynthetic and structural investment as well as biochemical properties of leaves (Griffith et al., 2016). As such, LT may represent a less costly trait to be modulated in comparison with LDMC, as already suggested (de la Riva et al., 2016; Puglielli and Varone, 2018). Accordingly, we found that LDMC changes accounted for most of the variance in Am reflecting that modulating LDMC is costly in terms of carbon assimilation compared to LT adjustments. Moreover, changes in LMA as a function of LDMC (i.e. slopes) were significantly related to %C further demonstrating that the adjustment of LMA–LDMC relationship is directly involved in shaping resource acquisition strategies with varying environmental conditions in *Cistus* spp. However, we want to stress that besides the morphological control on the photosynthetic process there are of course underlying physiological processes to be also considered as players in such strategy and nitrogen re-translocation could be one of the candidates (see Puglielli and Varone, 2018 for further discussion but also Milla et al., 2004; Simões et al., 2012; Dias et al., 2012; Correia and Ascensão, 2017). In depth analysis of leaf life span changes between leaf cohorts during a growing season are also needed.

5. Conclusions

In conclusion, the results strongly support that *Cistus* spp. can adapt leaf structure–function relationships in leaves formed under prevailing environmental conditions. This extends the current knowledge on *Cistus* spp. leaf cohorts structuring and functioning highlighting that the broad classification in winter and summer leaves is not able to capture the entire seasonal spectrum of trait variations and covariations in *Cistus* spp. In fact, we found that *Cistus* spp. employ different strategies to cope with climate variability during the investigated growing season. As such, even if general patterns of traits covariation were found on pooled data, leaves formed under different environmental conditions can differ from such patterns.

Overall, it is evident that winter and summer leaves represent the extremes of the seasonal spectrum of strategies employed by *Cistus* spp. to cope with environmental changes, but it is noteworthy that discrete intermediate strategies can exist. Based on our results, these strategies are mainly employed by the independent modulation of LT and LDMC with varying environmental conditions which is constrained by the extent of control that LT and LDMC exert on the photosynthetic process. However, integration of such evidence with nitrogen re-translocation and high resolution leaf life span data are needed in future studies in order to fully understand the mechanisms behind these strategies. Our results call for the necessity to review the generally accepted winter–summer leaf classification reported in literature and provides a new framework to better describe *Cistus* spp. response to environmental changes. This is particularly relevant considering that functional differences among leaf flushes formed at different times during a growing season are expected to increase due to global climate change (Niinemets, 2014). Moreover, considering the role of *Cistus* spp. in shaping structure and function of the Mediterranean ecosystems (de Dato et al., 2013; Correia and Ascensão, 2017), the understanding of this fine ability to modulate leaf structure–function relationships can help to better predict *Cistus* species' response to the major drivers of global climate change.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.flora.2018.08.010>.

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